Program/Abstract # 311
Role of Wnt4 in chick myogenesis
Anthony S. Eritano, Lisa M. Galli, Laura W. Burrus
Department of Biol., SFSU, San Francisco, CA, USA

The Wnt family is comprised of 20 highly conserved signaling proteins that control proliferation, specification, differentiation and survival. Several lines of evidence have implicated Wnt4 in myogenesis. For instance, the expression of Wnt4 in the neural tube immediately precedes expression of Myf5 and MyoD in the medial somite. Furthermore, addition of Wnt4 to presomitic mesoderm explants causes an upregulation of Pax3 and Pax7 (Fan et al, Dev Biol, 1997). Ectopic Wnt4 also causes differentiation of C2C12 myoblasts into muscle cells (Takata et al, Dev Dyn, 2007), thus further implicating Wnt4 as a possible promoter of myogenesis. Based on these studies, I hypothesized that Wnt4 acts to promote the differentiation of myogenic precursor cells into muscle. To test my hypothesis, I overexpressed Wnt4 in the chick neural tube via electroporation and analyzed sections that were stained for myosin heavy chain (MHC). Consistent with my hypothesis, staining for MHC revealed a statistically significant 1.2 fold increase in the area of the myotome as compared to control embryos that were electroporated with a construct expressing GFP alone. The increase in myotome size could indicate either cell hypertrophy or an influx of myogenic precursor cells from the dermomyotome. To distinguish between these two possibilities, I quantitated the size of cells in the myotome and the total number of cells/nuclei in the myotome. Immunostaining with b-catenin antibodies enabled me to monitor cells size while staining with DRAQ5 (a DNA stain) allowed me to quantitate the number of nuclei. My results show that the increase in the size of the myotome is due to cellular hypertrophy and not an increase in the number of cells.

doi:10.1016/j.ydbio.2009.05.339

Program/Abstract # 312
Intraembryonic functions of IGF2 signaling in zebrafish
Antony W. Wood, Yvonne A. Brown
Vincent Center for Reproductive Biology, Massachusetts General Hospital, Boston, MA, USA

Insulin-like growth factor 2 (IGF2) is the predominant IGF ligand regulating prenatal growth in all vertebrates, but its central role in placental development has confounded efforts to elucidate its functions within the embryo. Here, we use a non-placental vertebrate (zebrafish) to interrogate the intraembryonic functions of IGF2 signaling. Zebrafish have two *IGF2* co-orthologs (*igf2a*, *igf2b*), which exhibit distinct patterns of expression. *igf2a* mRNA is expressed in the notochord, primarily during segmentation/neurulation, whereas *igf2b* mRNA is expressed in midline tissues adjacent to the notochord, with additional expression in the ventral forebrain and the pronephros. To identify their intraembryonic functions, the expression of each gene was suppressed with morpholinos. Knockdown of *igf2a* led to defects in dorsal midline development, characterized by delayed segmentation, notochord undulations, and ventral curvature. Suppression of *igf2b* led to similar defects in dorsal midline development, but also induced ectopic fusion of the nephron primordia, and defects in ventral forebrain development. Simultaneous knockdown of both genes increased the severity of dorsal midline defects, confirming a conserved role for both genes in dorsal midline development. Subsequent onset of severe whole-body edema in *igf2b*, but not *igf2a* morphants, confirmed a distinct role for *igf2b* in development of the embryonic kidney. Collectively, these data provide evidence that the zebrafish orthologs of *IGF2* function in dorsal midline development during segmentation/neurulation, while one paralog, *igf2b*, has evolved additional, distinct functions during subsequent organogenesis.

doi:10.1016/j.ydbio.2009.05.340

Program/Abstract # 313
C2cd3 is required for cilia formation and Hedgehog signaling in mouse
Aimin Liu[a], Aaron Wynkoop[a], Huiqing Zeng[a], Jinping Jia[a], Lee Niswander[b], Aimin Liu[a]
[a]*Department of Biol., Penn State Univ, University Park, PA, USA*
[b]*Department of Pediatrics, UCHSC, Aurora, CO, USA*

Cilia are essential for mammalian embryonic development as well as for the physiological activity of various adult organ systems. Despite the multiple crucial roles that cilia play, the mechanisms underlying ciliogenesis in mammals remain poorly understood. Taking a forward genetic approach, we have identified Hearty (Hty), a recessive lethal mouse mutant with multiple defects, including neural tube defects, abnormal dorsal–ventral patterning of the spinal cord, a defect in left–right axis determination and severe polydactyly (extra digits). By genetic mapping, sequence analysis of candidate genes and characterization of a second mutant allele, we identify *Hty* as *C2cd3*, a novel gene encoding a vertebrate-specific C2 domain-containing protein. Target gene expression and double-mutant analyses suggest that *C2cd3* is an essential regulator of intracellular transduction of the Hedgehog signal. Furthering a link between Hedgehog signaling and cilia function, we find that cilia formation and proteolytic processing of Gli3 are disrupted in *C2cd3* mutants. Finally, we observe C2cd3 protein at the basal body, consistent with its essential function in ciliogenesis. Interestingly, the human ortholog for this gene lies in proximity to the critical regions of Meckel–Gruber syndrome 2 (MKS2) and Joubert syndrome 2 (JBTS2), making it a potential candidate for these two human genetic disorders.

doi:10.1016/j.ydbio.2009.05.341

Program/Abstract # 314
Tbx1 regulates mesenchymal–epithelial signaling necessary for inner ear development
Dennis C. Monks, Evan M. Braunstein, Bernice E. Morrow
Department of Genetics, Albert Einstein College of Medicine, Bronx, NY, USA

The transcription factor *Tbx1* is a member of the T-box family and its deletion is responsible for velo-cardio-facial/DiGeorge syndrome. *Tbx1*−/− mice show severe developmental defects, including missing outer, middle, and inner ear structures. *Tbx1* is expressed in two regions critical for inner ear development: the epithelium of the otic vesicle (OV) and the surrounding periotic mesenchyme (POM). Conditional inactivation of *Tbx1* in the OV mimics the null phenotype, whereas inactivation in the POM yields a phenotype with decreased proliferation and survival of the OV epithelium, failed cochlear outgrowth, and a hypoplastic vestibular system. This suggests that mesenchymal *Tbx1* regulates pathways necessary for proper survival and development of the OV. While the importance of epithelial-to-mesenchymal signaling during inner ear development has been well established, signaling from the POM to the OV has not been well studied. To elucidate signaling pathways downstream of mesenchymal *Tbx1*, we have utilized both candidate gene and microarray approaches. Our data suggest that retinoic acid is one candidate pathway downstream of mesenchymal *Tbx1*. Additionally, BMP signaling, specifically the BMP inhibitor *Follistatin*, was identified by